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# Indications of future performance of native and non-native adult oysters under acidification and warming

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## Abstract

Globally, non-native species (NNS) have been introduced and now often entirely replace native species in captive aquaculture; in part, a result of a perceived greater resilience of NSS to climate change and disease. Here, the effects of ocean acidification and warming on metabolic rate, feeding rate, and somatic growth was

assessed using two co-occurring species of oysters – the introduced Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*), and native flat oyster *Ostrea edulis*. Biological responses to increased temperature and  $p\text{CO}_2$  combinations were tested, the effects differing between species. Metabolic rates and energetic demands of both species were increased by warming but not by elevated  $p\text{CO}_2$ . While acidification and warming did not affect the clearance rate of *O. edulis*, *M. gigas* displayed a 40% decrease at ~750 ppm  $p\text{CO}_2$ . Similarly, the condition index of *O. edulis* was unaffected, but that of *M. gigas* was negatively impacted by warming, likely due to increased energetic demands that were not compensated for by increased feeding. These findings suggest differing stress from anthropogenic  $\text{CO}_2$  emissions between species and contrary to expectations, this was higher in introduced *M. gigas* than in the native *O. edulis*. If these laboratory findings hold true for populations in the wild, then continued  $\text{CO}_2$  emissions can be expected to adversely affect the functioning and structure of *M. gigas* populations with significant ecological and economic repercussions, especially for aquaculture. Our findings strengthen arguments in favour of investment in *O. edulis* restoration in UK waters.

**Keywords:** climate change; ecosystem change; exotic species; living resources; oyster; physiology; UK

## 45 Introduction

46 Ocean acidification and warming (OAW) affects the behaviour, metabolism, and  
47 performance of a diversity of marine organisms (Barry *et al.*, 2011; Kroeker *et al.*,  
48 2013). Early-life history stages, especially important in population persistence, are  
49 shown to be particularly vulnerable (Byrne & Przeslawski, 2013; Kurihara, 2008;  
50 Przeslawski *et al.*, 2015), and is raising concerns for the continued provision of  
51 important ecosystem services (Lacoue-Labarthe *et al.*, 2016; Lemasson *et al.*, 2017;  
52 Sunday *et al.*, 2016; Weatherdon *et al.*, 2016). Calcifying species are especially at  
53 risk as they are susceptible to alterations in ocean chemistry (Hofmann *et al.*, 2010;  
54 Parker *et al.*, 2013; Pörtner *et al.*, 2014), manifested by increased metabolism,  
55 respiration and energy expenditure (Pörtner & Farrell, 2008).

56

57 Species most resilient to OAW may well be those best able to enhance their energy  
58 assimilation. A common way for marine organisms to balance their energy intake  
59 and expenditure is to increase their feeding rate, (Ramajo *et al.*, 2015; Sanders *et al.*,  
60 2013; Thomsen *et al.*, 2012; Towle *et al.*, 2015) or reallocate energy through  
61 partitioning and trade-offs between reproduction, somatic growth and calcification  
62 (Leung *et al.*, 2017). Species less able to manipulate their feeding activity to offset  
63 stress from OAW may show reduced energetic levels and capacity for metabolic  
64 maintenance (Houlbrèque *et al.*, 2015; Mackenzie *et al.*, 2014; Vargas *et al.*, 2015).  
65 OAW may therefore be an important selection pressure that dictates the distribution  
66 of species and functioning of marine ecosystems. Today, there is pressure to  
67 understand the effects of OAW on species that provide important ecosystem goods

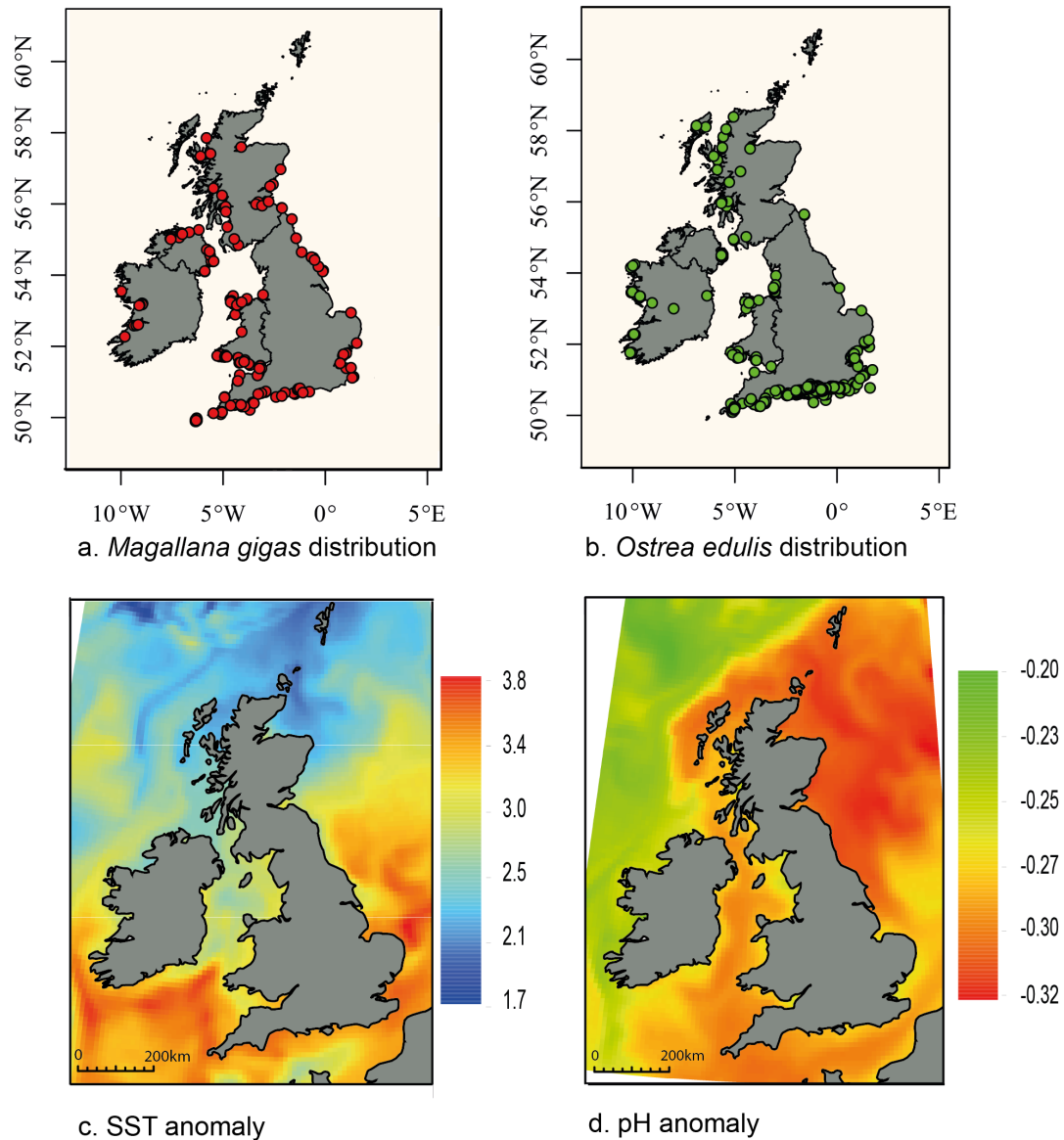
and services (Osborn *et al.*, 2017) and mitigate negative impacts of OAW to ensure the sustainable delivery of the services derived from those species in to the future.

In the UK, the native European flat oyster, *Ostrea edulis*, and the non-native Pacific oyster, *Magallana gigas* (which until recently was named *Crassostrea gigas*) are two valuable commercially-exploited species. They provide relatively similar and numerous ecosystem services (Herbert *et al.*, 2012) including: reef formation, erosion control, improvement of water quality (through cycling and purification), raw material supply, and food provision (through aquaculture and fisheries) (see Coen *et al.*, 2007, for a review of oyster-associated ecosystem services; Herbert *et al.*, 2012). Historically, *O. edulis* was highly abundant and was the basis of a major shellfish fishery in the UK and Europe (Coolen, 2017; Orton, 1937), but today is a protected species in the UK with active restoration efforts underway to counteract ever declining stocks from overharvesting, competition, pests, diseases, and reproductive failures (Laing *et al.*, 2006; Lallias *et al.*, 2010; Woolmer *et al.*, 2011). In contrast, *M. gigas* was introduced to the UK within regulated aquaculture settings in the mid-20<sup>th</sup> Century in response to the decline of *O. edulis*, and today this species represents over 90% of UK oyster aquaculture production, worth an estimated £10.14 million annually (Humphreys *et al.*, 2014).

*Magallana gigas* was originally introduced under the assumption that local seawater temperatures would prevent its reproduction and the formation of viable wild populations, nonetheless the species has formed unintended wild populations on UK and Irish shores where it is often considered invasive (Dolmer *et al.*, 2014; Herbert *et al.*, 2016; Kochmann *et al.*, 2013; Troost, 2010). Despite the occurrence of wild

93 populations, the harvest of *M. gigas* is currently mostly limited to regulated  
94 aquaculture sites (Herbert *et al.*, 2012). Today, beds comprised of both *M. gigas* and  
95 *O. edulis* occur, such as in Ireland (Zwerschke *et al.*, 2017) and at sites along the  
96 South-West coast of the UK (pers. observations; Fig 1.a,b). It is often speculated that  
97 *M. gigas* and *O. edulis* compete for space and resources, with the presence of  
98 *M. gigas* having negative consequences for *O. edulis*, although there is no  
99 documented evidence of this. In fact, a recent study suggests no evidence of  
100 competition between the two species (Zwerschke *et al.*, 2016). Nevertheless, the  
101 negative perception of wild *M. gigas* populations has led to management measures  
102 being introduced to prevent its further proliferation, and to promote the recovery of  
103 *O. edulis* (Harding *et al.*, 2016; Herbert *et al.*, 2012; Laing *et al.*, 2006; Sawusdee,  
104 2015; Woolmer *et al.*, 2011).

105



**Fig 1. Current UK wild distribution of (a) *Magallana gigas* (red) and (b) *Ostrea edulis* (green) (data obtained from the Global Biodiversity Information Facility (GBIF) database), (c) maximum mean annual sea surface temperature (SST) anomaly (SST, in °C; medium emission scenario IPCC SRES: A1B for 2070-2099, data obtained from UKCP09) and (d) minimum mean annual surface water pH anomaly (scenario for 2080-2099, data obtained from the Marine Ecosystem Evolution in a Changing Environment (MEECE) database).**

114

115 Since its introduction to Europe, *M. gigas* has been spreading northward across  
116 European shores (Shelmerdine *et al.*, 2017) facilitated by increasing average sea  
117 surface temperatures (SST) (Angles d'Auriac *et al.*, 2017; Rinde *et al.*, 2016;  
118 Thomas *et al.*, 2016; Townhill *et al.*, 2017). In contrast, the extent of *O. edulis* is  
119 continuing to decline, and native oyster reefs are considered some of the most  
120 endangered coastal habitats in Europe (Airoldi & Beck, 2007; Beck *et al.*, 2011). The  
121 success of introduced species is often attributed to their greater tolerance (and  
122 physiological plasticity) to fluctuating environmental conditions than their native  
123 counterparts (Hall-Spencer & Allen, 2015; Lodge, 1993; Stachowicz *et al.*, 2002). For  
124 example, in Australia, early-life stages of *M. gigas* (introduced) were shown to be  
125 less sensitive to OAW than the native *Saccrostrea glomerata* (Parker *et al.*, 2010)  
126 and in Brazil, introduced *M. gigas* was more resilient to extreme hypercapnic  
127 conditions than the native *Crassostrea brasiliiana* (Moreira *et al.*, 2018). A similar  
128 response has also been shown in other taxa. For example, in Spain, the non-  
129 indigenous mussel *Xenostrobus securis* was found more resilient to reduced pH than  
130 the native *Mytilus galloprovincialis* (Gestoso *et al.*, 2016). This precedent would  
131 suggest it is not unreasonable to expect *M. gigas* to display similar tolerance in the  
132 UK, and be more resilient than its native counterpart *O. edulis* to future change in  
133 environmental conditions.

134

135 As calcifiers, both oyster species can be expected to be negatively impacted by  
136 ocean acidification. The risks that ocean acidification pose to oysters were first  
137 highlighted in 2007 when hatcheries in the Pacific North-West region of the US  
138 suffered mass mortalities of Pacific oyster larvae. Upwelling of acidified water with



139 low aragonite saturation (a principle biomineral used in shell maintenance) caused  
140 an 80% reduction in hatchery production and significant financial losses (Barton *et*  
141 *al.*, 2015; Cooley *et al.*, 2017). Since then, studies into the effects of OAW on oysters  
142 and other commercially important bivalves have rapidly increased in number.  
143 Extensive work has been done on early life stages, demonstrating sensitivity to OAW,  
144 but also other environmental stressors (Cole *et al.*, 2016; Parker *et al.*, 2017a).  
145 Responses include slower calcification (Waldbusser *et al.*, 2016), delayed growth,  
146 and delayed or abnormal development (Gray *et al.*, 2017; Parker *et al.*, 2010;  
147 Waldbusser *et al.*, 2015).  
148  
149 Less work has been undertaken on juveniles and adults, although impacts on early  
150 life stages has been shown to “carry-over” into these life-history stages (Hettinger *et*  
151 *al.*, 2013b; Hettinger *et al.*, 2012). Both juveniles and adults have shown altered  
152 immune response (Liu *et al.*, 2016; Wang *et al.*, 2016), reduced calcification and  
153 shell growth (Beniash *et al.*, 2010; Waldbusser *et al.*, 2011b; Wright *et al.*, 2014),  
154 increased shell dissolution (Waldbusser *et al.*, 2011a), and reductions in shell  
155 strength (Dickinson *et al.*, 2012; Mackenzie *et al.*, 2014; Welladsen *et al.*, 2010).  
156 Crucial metabolic activities, such as respiration and feeding, can also be impacted  
157 (Comeau *et al.*, 2008; Dove & Sammut, 2007; Scanes *et al.*, 2017), the resulting  
158 stress likely leading to mortality and reduced population resilience, impaired  
159 biological functioning, and reduced ecosystem service provision (Lemasson *et al.*,  
160 2017).  
161  
162 Temperature is considered a major determinant of species and ecosystem structure  
163 and functioning. For *M. gigas*, its thermal range is reported as 1.8-35°C (see FAO

factsheet; Fig 1.a). While the thermal optima is not known for this species (and may vary as a result of local adaptation, see Sanford & Kelly, 2011), given its evolutionary origins, it is argued that in the UK, increasing average SST that is associated with climate change allows increased metabolic performance, individual growth, and range expansion. For *O. edulis*, the thermal range is less well defined and where data are available, the evidence is contradictory (Shelmerdine & Leslie, 2009). In one instance, temperatures higher than 20°C have been shown to be suboptimal, negatively affecting growth, metabolism and filtration activity in juvenile *O. edulis* (Buxton *et al.*, 1981), but conversely, cold has also been shown to limit larval production, recruitment, and growth below temperatures of 17.5°C (Beiras *et al.*, 1995; Davis & Calabrese, 1969; Orton, 1940; Robert *et al.*, 2017; Walne, 1958). Differences in response may be related to dispersal capacity. *Magallana gigas* generate solely planktotrophic larvae, whereas *O. edulis* first brood (larviparous) before generating shorter planktonic duration planktotrophic larvae, which arguably limits dispersal capacity and promotes a greater likelihood of local adaptation (Bertness & Gaines, 1993) in *O. edulis* over *M. gigas* making developmental performance thresholds less clear.

It is therefore unclear how continued CO<sub>2</sub> emissions and associated increases in ocean acidification and warming will affect wild and harvested populations of *M. gigas* and *O. edulis* in the UK, nor what the consequences for ecological functioning and provisioning of ecosystem services will be. Substitution of one species for another, either partially or entirely, can produce significant ecological impacts (Krasso *et al.*, 2008), but since *M. gigas* is, in theory, able to provide similar ecological functions and ecosystem services as *O. edulis* (Herbert *et al.*, 2016;

Zwerschke *et al.*, 2016) and is currently present in higher abundances, efforts to eradicate it may be unwise if it becomes increasingly prevalent under climate change.

In this study, we test the effects of OAW on the physiological responses of a native and a non-native species of UK oyster to determine the potential respective ecosystem service contribution of these species both today and in the future. Individual measures of fitness were assessed using Standard Metabolic Rate (SMR), Clearance Rate (CR), and Condition Index (CI) under simulated warming and acidification scenarios over a 12 week period. SMR was used as a proxy for metabolic costs and energetic requirements, while CR informed us of energy uptake. CI was used to assess overall health and quality and the availability of energy reserves within somatic tissues. Our hypotheses were that future OAW conditions would induce metabolic costs for both species of oysters, along with compensatory increases in energy acquisition through enhanced feeding. Additionally, we hypothesised that *M. gigas* would show evidence of higher tolerance to warming and acidification than *O. edulis*.

## Methods

### Organism collection and acclimation

Adult Pacific oysters (*M. gigas*;  $112.4 \pm 6.9$  mm in length and weighing  $285.9 \pm 13.4$  g), and European flat oysters (*O. edulis*;  $79.4 \pm 5.7$  mm in length and weighing  $92.8 \pm 15.1$  g) were hand-collected from a wild population at a low-intertidal fully marine site in Plymouth Sound, UK ( $50^{\circ}23'29.95''\text{N}$ ,  $004^{\circ}13'16.77''\text{W}$ ), in July 2015 and January 2016, respectively. Oysters were cleaned of epibionts and allowed to

acclimatise in a recirculating system to ambient laboratory conditions of ~16.5°C and atmospheric pressure of 400ppm at the University of Plymouth (UK). Over an acclimation period of 14 days, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture).

## **Experimental design**

Following acclimation to laboratory conditions, 24 oysters were placed in their own 3 L experimental tank (four tanks per OAW scenario) and exposed to the treatment conditions. Three levels of  $p\text{CO}_2$  (ambient 400 ppm, intermediate 750 ppm, elevated 1000 ppm), and two temperatures (control 16.8 °C, elevated 20 °C), were tested in an orthogonal experimental design to simulate current and future OAW scenarios. These six scenarios are in line with warming and acidification conditions predicted for the UK (Fig 1c,d). As such, temperature scenarios reflected maximum current SST (16.8°C), and predicted SST for the end of the century (20°C, corresponding to the predicted increase by 3-4°C in average SST along the South-West of the UK). However, it should be noted that such predictions do not taken into account localized variability in environmental conditions often experienced by organisms in coastal and estuarine habitats, and which may be amplified by future OAW. Due to capacity limitations of our mesocosm system, the experiment ran for 12 weeks between September and November 2015 for *M. gigas*, then repeated with *O. edulis* following the same procedures between January and March 2016. As such, the environmental conditions experienced by each species were inherently different due to natural seasonal variations in seawater properties driven by differences in atmospheric conditions (e.g. barometric pressure). The resulting pH conditions were therefore

different between experiments (Fig 2, S1 Table), but the effect size (magnitude of difference in pH between experimental treatments) were comparable.

## **Mesocosm set-up**

The ocean acidification and warming mesocosm system used during the experiment is a modified version of the one described by Calosi *et al.*, (2013). Briefly, each treatment consisted of a header tank (volume=80 L) of seawater, supplied from one of two sumps (16.5 °C and 20 °C), and aerated with either the ambient air pipe ( $p\text{CO}_2$  400 ppm) or one of the two  $\text{CO}_2$ - enriched air pipes ( $p\text{CO}_2$  750 ppm,  $p\text{CO}_2$  1000 ppm). Ambient air consisted of laboratory air subjected to diurnal variability. Mixing in all header tanks was achieved using a submersible pump (Hydor Koralia Nano 900, Italy).  $\text{CO}_2$  gas mix were obtained by slowly releasing  $\text{CO}_2$  into two Buchner flasks where it mixed with ambient air, achieving two different levels of  $p\text{CO}_2$ , using multistage  $\text{CO}_2$  regulators (EN ISO 7291; GCE, Worksop, UK). As such, throughout the experiment the three  $\text{CO}_2$  levels varied in a similar manner following natural variations in  $\text{CO}_2$  in the ambient air. The treatments thus took account of natural daily variability, which has been suggested as a critical consideration for climate change experimental studies (Humphreys, 2016; Reum *et al.*, 2015).  $\text{CO}_2$  levels in the two  $\text{CO}_2$ -enriched pipes were recorded using a  $\text{CO}_2$  analyser (LI-820; LI-COR, Lincoln, NE, USA) and adjusted manually to the desired level twice daily.  $\text{CO}_2$  levels in the ambient air pipe were also recorded to monitor the levels of the control treatments. Seawater was gravity-fed from the header tanks to each of the corresponding replicate tanks (3 L transparent sealed containers) at a constant rate of ~60 mL/min. The replicate tanks were held within four larger 300 L holding trays, each sump supplying seawater to two of the holding trays, effectively creating water

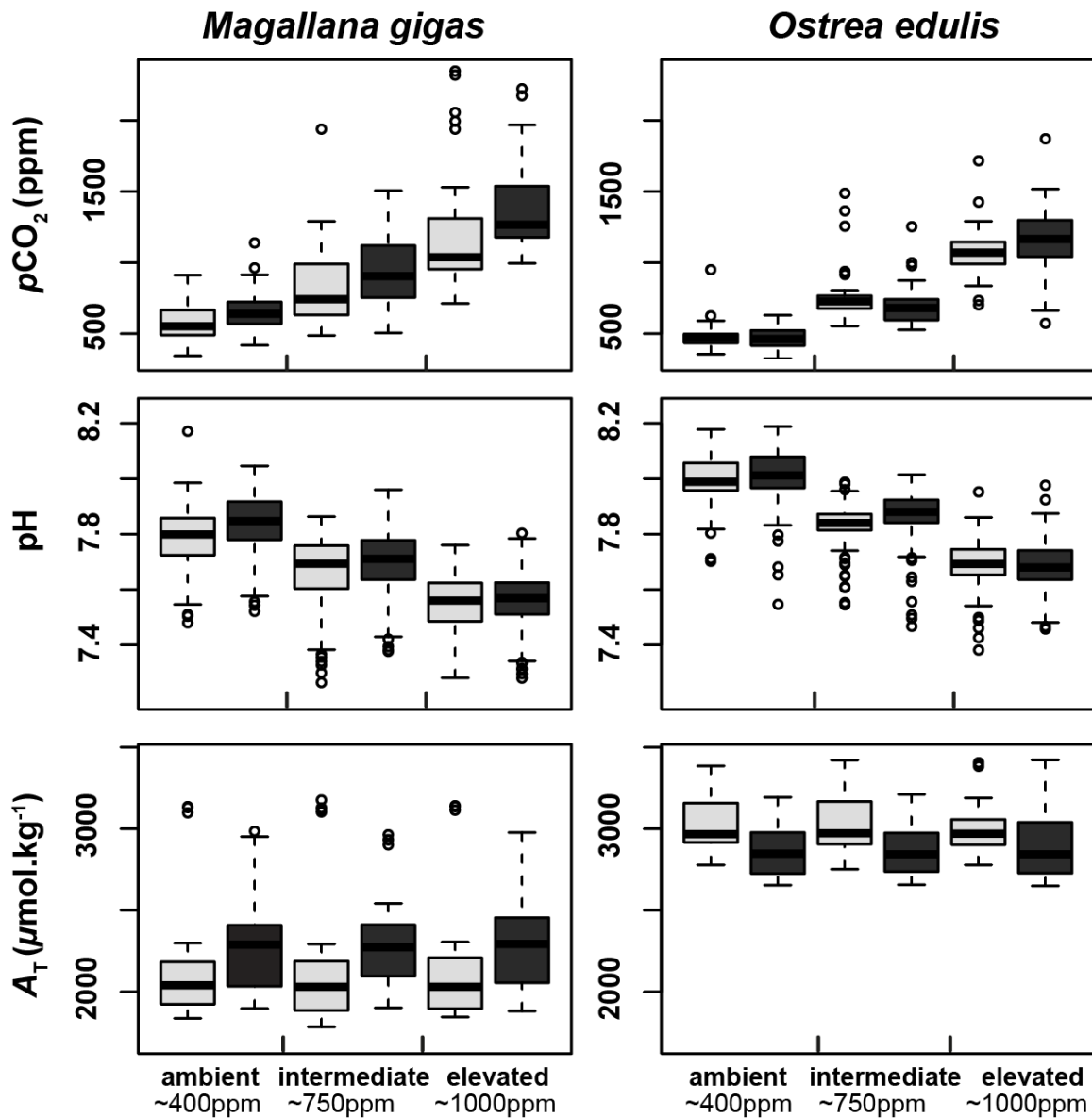
baths maintaining the replicate tanks at the desired temperature (two water baths at 16.5°C, two water baths at 20°C). Each tray held two replicates of each CO<sub>2</sub> levels (four replicates per temperature and CO<sub>2</sub> treatment). Excess seawater was allowed to overflow from the trays to their corresponding sump, where it was filtered, aerated, and recirculated to the corresponding header tanks and trays using a submersible pump (1262; EHEIM GmbH and Co. KG, Deizisau, Germany). Seawater in the system originated from Plymouth Sound (UK) and, following mechanical filtering and UV sterilization, was added and replaced on a daily basis to account for evaporation, Deionized water was added as needed to maintain stable salinity levels. In elevated temperature treatments, seawater was increased to 20°C using aquarium heaters (50 W aquarium heater; EHEIM Jager GmbH and Co. KG, Stuttgart, Germany) placed in header tanks and holding trays.

## **Measurements of seawater parameters**

Temperature, salinity, and pH were measured daily in all replicate tanks (Fig 2. see also S1 Table and S1 Fig. for details of temperature and pH data). Salinity was measured using a handheld refractometer (D&D The Aquarium Solution Ltd, Ilford, UK) and temperature measured using a digital thermometer (TL; Fisher Scientific, Loughborough, UK). pH was measured using a microelectrode (InLab® Expert Pro-ISM; Mettler-Toledo Ltd, Beaumont Leys, UK) coupled to a pH meter (S400 SevenExcellence™; Mettler-Toledo Ltd, Beaumont Leys, UK), following calibration with NIST traceable buffers. pH in the header tanks was also monitored (data not shown). Total Alkalinity (A<sub>T</sub>) was measured once a week in each of the replicate tanks. 125 mL water samples were transferred to borosilicate bottle with Teflon caps and poisoned with 30 µL of saturated HgCl<sub>2</sub> solution (0.02 % sample volume) before

being kept in the dark until measurement by automatic Gran titration (Titralab AT1000 © Hach Company). Partial pressure of carbon dioxide ( $p\text{CO}_2$ ) and saturation states of calcite and aragonite ( $\Omega_{\text{calcite}}$  and  $\Omega_{\text{aragonite}}$ ), were calculated at the end of the experiment using CO2 SYS (Pierrot *et al.*, 2006), employing constants from Mehrbach *et al.* (1973) refitted to the NBS pH scale by Dickson and Millero (1987) and the  $\text{KSO}_4$  dissociation constant from Dickson (1990) (Fig 2., see also S1 Table).

Throughout the duration of the experiment, oysters were fed daily with 20 mL of a live algae (mixed diet of *Isochrysis galbana* and *Tetraselmis* sp.) to obtain a concentration of approximately  $10^8 \text{ cell.L}^{-1}$  within the experimental tank. Three times a week, tanks were gently brushed and siphoned to remove faeces and excess food, thereby insuring acceptable water quality, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater.



**Fig 2. Variation in  $p\text{CO}_2$ , pH, and total alkalinity ( $A_T$ ), of seawater in the experimental treatments.** ppm=part per million. Grey= control temperature (~16.8°C, black= elevated temperature (~20.0°C). Data are pooled based on daily (pH) and weekly ( $A_T$ ) measurements over the 12 week experimental duration. Weekly  $p\text{CO}_2$  values were calculated using CO2 SYS (Pierrot D *et al.*, 2006).

## Physiological measurements



Following 10 days, 5, 9, and 12 weeks of exposure to each OAW scenario, metabolic activity and energy acquisition were measured for each oyster (N=24 per species; 4 per OAW scenario). To limit post-prandial metabolism of food and excretion of faeces that could alter the results, oysters were not fed for 24h prior to measurements.

### Standard metabolic rate

Respiration rates were measured as proxy for Standard Metabolic Rates (SMR), using microfiber optic oxygen sensors (Firebox 4, PreSens Germany, [www.presens.de](http://www.presens.de)). All oysters (N=24 per species; 4 per OAW scenario) was placed in a 1.2 L air-tight container, filled with 1 L of seawater filtered to 2  $\mu\text{m}$  and pre-equilibrated to their respective experimental  $p\text{CO}_2$  and temperature treatment. To maintain stable temperature in the chambers, all measurements were conducted in controlled-temperature rooms. The seawater in each chamber was stirred using a magnetic rod for the duration of the assay (350 rpm). Respiration measurements started when the oyster resumed filtration, and ended either when  $\text{O}_2$  saturation reached 80% to prevent the organisms from experiencing hypoxic conditions, or when the oyster shut its valves.  $\text{O}_2$  measurements were corrected for temperature, salinity, and barometric pressure using Green and Carritt's (1967) oxygen solubility coefficients and Weiss' (1970) vapour pressure values, as well as corrected for background bacterial respiration (the reduction in dissolved oxygen in each tank without shellfish was subtracted from total  $\text{O}_2$  reductions in the same tank with shellfish) and the individuals' volume and dry weight, to obtain absolute quantities of oxygen consumed. Temperature and salinity was recorded at the start of each assay as described above. Barometric pressure data were obtained from the Plymouth Live

Weather Station (<http://www.bearsbythesea.co.uk>). Dry weight was assessed at the end of the 12-wk exposure (see below “Condition Index” section for details). Volume was determined using the water displacement method. SMR was calculated as follows:

$$SMR = \frac{V_r(L) \times \Delta C_w O_2 (mg O_2 \cdot L^{-1})}{\Delta t(h) \times bw(g)} [1]$$

where SMR is the oxygen consumption normalized to 1 g of dry tissue mass (DW) in  $mg O_2 \cdot g^{-1} DW \cdot h^{-1}$ ;  $V_r$  is the volume of the respirometry chamber minus the volume of the oyster (L);  $\Delta C_w O_2$  is the change in water oxygen concentration measured ( $mg O_2 \cdot L^{-1}$ );  $\Delta t$  is measuring time (h); and  $bw$  is the dry tissue mass (g) of the oyster.

### Clearance rates

Directly following the respirometry assay, the Clearance Rate (CR) of all oysters from each treatment (n=4) was calculated using methods previously described in Coughlan (1969) and Sanders *et al.*, (2013). Individuals selected for clearance rate measurements were the same individuals used for the respirometry assay described above. Oysters were placed in a 1.2 L chamber, filled with 1 L of seawater filtered to  $2 \mu m$  and pre-equilibrated at their respective experimental  $pCO_2$  and temperature treatment. To maintain stable temperature in the chambers, all measurements were conducted in controlled-temperature rooms. ~20 mL of the same live algae culture (mix of *Tetraselmis* sp. and *Isochrysis galbana*) was added to each chamber when oysters started filtering. To allow homogeneous mixing of algae, the seawater in each chamber was stirred using a magnetic rod (350 rpm). Three replicate 5mL water samples were taken from haphazard locations throughout the chamber (1) prior to the addition of food ( $t_i$ ); (2) immediately after addition of food ( $t_0$ ) to check the

initial algal concentration; and (3) at 10 minute intervals following food addition for a duration of 40 minutes, providing 6 sampling times (i.e.  $t_i$ ,  $t_o$ ,  $t_1$ ,  $t_2$ ,  $t_3$ , and  $t_4$ ). If the oyster shut its valves, the chronometer was stopped and restarted once the valves re-opened. Counts of algae in all water samples were performed in triplicate using a Coulter Counter (Beckman Coulter Z2). Clearance rates (CR) were calculated using the following equation after Coughlan (1969):

$$CR = \frac{V \times \ln\left(\frac{C_{n-1}}{C_n}\right)}{t_n - t_{n-1}} [2]$$

where CR is the clearance rate measured during the 10 minute interval between sampling times  $t_{n-1}$  and  $t_n$ , normalized to 1 g of dry tissue mass ( $L^{-1}.g^{-1}DW.h^{-1}$ ),  $V$  is the volume of the chamber in L,  $C_{n-1}$  is the concentration ( $cell.L^{-1}$ ) in the sample taken at time  $t_{n-1}$  (hour), and  $C_n$  is the concentration ( $cell.L^{-1}$ ) in the sample taken at time  $t_n$  (hour). Results are presented as CRmax, the maximum clearance rate observed during the 40-minute incubation.

## Condition index

The Condition Index (CI) of oysters was calculated at the end of each experiment based on dry weight following the method recommended by Lucas and Beninger (1985) and described in equation [3]. Condition indices are useful tools widely used in the aquaculture sector to evaluate the overall quality and health of bivalves (Knights, 2012; Marin *et al.*, 2003). They reflect their ability to withstand adverse conditions (Marin *et al.*, 2003) by describing the quantity of organic tissue present (Bodoy *et al.*, 1986).

$$CI = \frac{\text{dry meat weight}}{\text{dry shell weight}} \times 100 [3]$$

Dry tissue weight was determined after each oyster was shucked using an oyster knife and oven-dried at 105°C until a constant mass was achieved.

## **Statistical analyses**

All data were tested for the assumption of homogeneity of variances, and where not met, data were transformed using logarithmic or square-root transformations. If after transformations assumptions were still not met, equivalent non-parametric tests were conducted. Differences were considered statistically significant if  $p < 0.05$ . All data were analysed using the public domain software *R* (version 3.2.5 R Core Team, 2016). Due to natural variations in the chemistry of the seawater used during the experiments and the partial pressure of ambient air used, the treatments applied to each species were not consistent, and therefore, species were not formally compared and data analysed separately.

## **SMR and CR**

SMR and CR data were analysed using linear mixed effects (lme) models with an autocorrelation argument (nlme package; see Zuur *et al.*, (2009)). ‘Temperature’ and ‘pCO<sub>2</sub>’ were considered as fixed factors to assess differences in species’ response to the treatments, and ‘Exposure’ (levels: 10 days, 5 wk, 9 wk, 12 wk) nested within ‘Replicate’ to partition differences due to individual oysters. If significant differences were present, *post-hoc* test was performed to assess differences between treatment levels (TukeyC and Multcomp packages). For each species, data were interrogated for the presence of fundamental relationships between the two physiological traits using the Pearson’s correlation test.

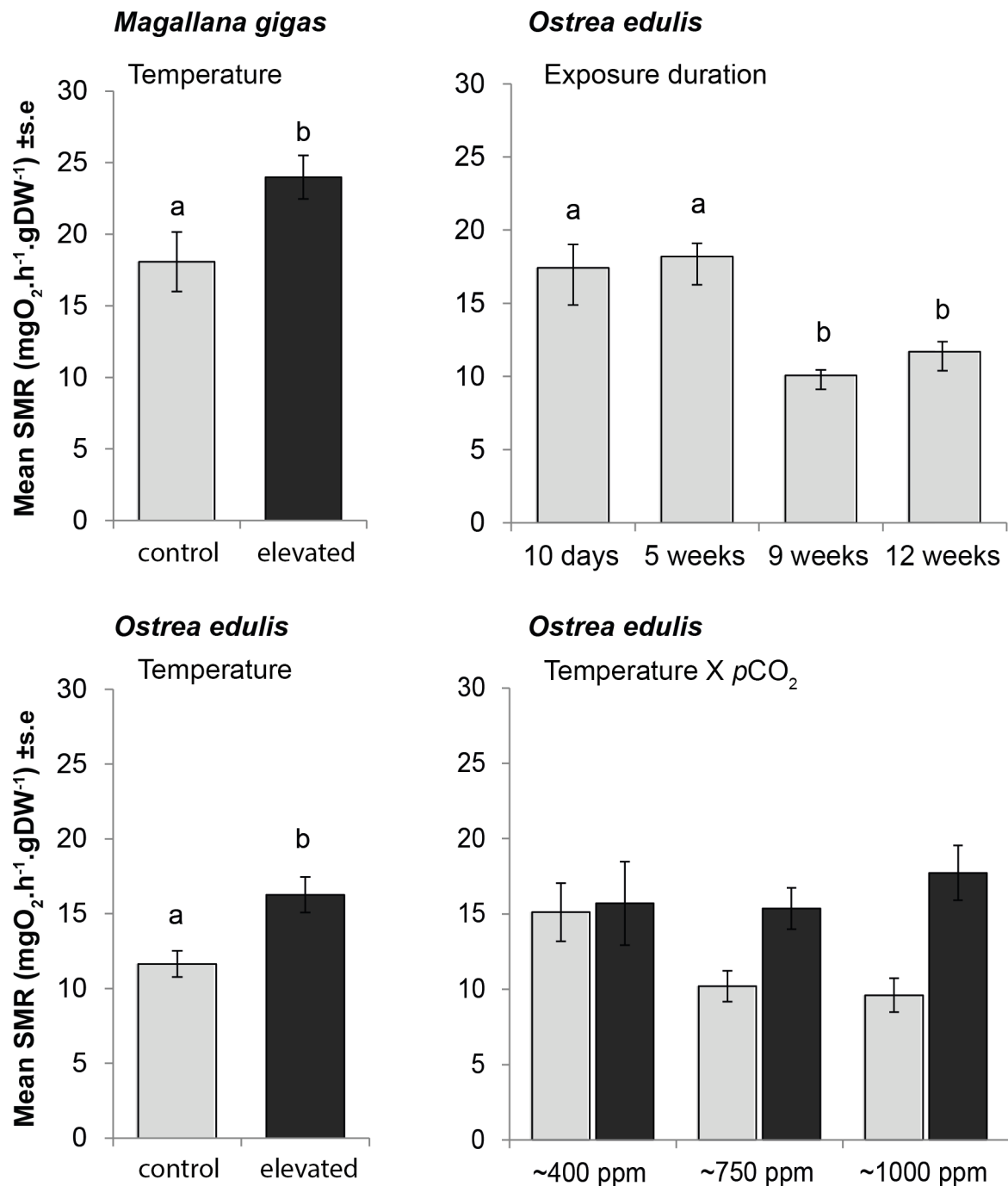
## Condition Index

Differences in CI with treatment were analysed using 2-factor ANOVA with 'temperature' (levels: 'control'; 'elevated') and ' $p\text{CO}_2$ ' (levels: 'ambient ~400ppm', 'intermediate ~750ppm', 'elevated ~1000ppm') as fixed factors. If significant differences were present, *post-hoc* pairwise comparisons (Tukey HSD) were performed to determine differences between treatment levels.

## **Results**

### **Standard metabolic rate**

For both species, there was clear inter-individual variability in responses (Fig 3.). The linear mixed-effects model revealed differences in metabolic response depending on exposure and OAW scenario S2 Fig.). For *M. gigas*, higher temperature, but not  $p\text{CO}_2$ , increased SMR by >43% (Fig 3.  $F_{1,18} = 11.51$ ,  $p < 0.01$ ). For *O. edulis*, exposure time led to a statistically significant decrease in SMR after 5 weeks ( $F_{1,71} = 25.55$ ,  $p < 0.001$ ), and temperature led to a statistically significant increase in SMR of >39% ( $F_{1,18} = 9.52$ ,  $p < 0.01$ ). However, it should be noted that for *O. edulis*, while the interaction between temperature and  $p\text{CO}_2$  was marginally not significant ( $F_{1,66} = 3.50$ ,  $p = 0.052$ ), clear trends were apparent. SMR decreased by up to 36% under elevated  $p\text{CO}_2$  conditions (750 and 1000 ppm) when oysters were kept at the control temperature, but when the temperature was elevated, there was no change in SMR even when  $p\text{CO}_2$  was increased. This was especially notable under 1000 ppm  $p\text{CO}_2$ , where SMR was ~46% lower in the control temperature than in the warm temperature treatment.



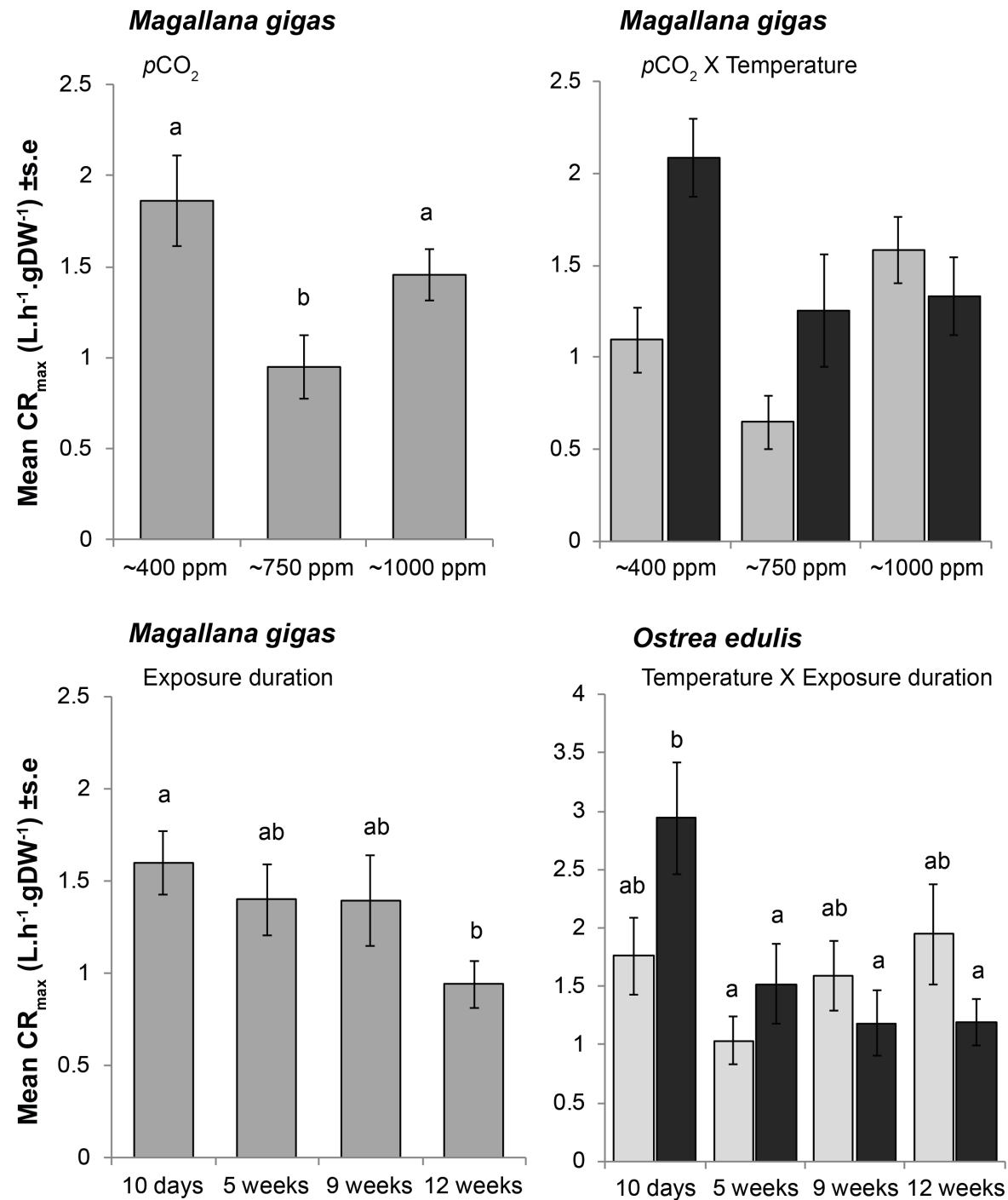
**Fig 3. Changes in standard metabolic rates (SMR) of *M. gigas* (top left) and *O. edulis* (bottom left) with temperature treatment; and of *O. edulis* with exposure duration (top right) and the interaction of temperature and pCO<sub>2</sub> treatments (bottom right). Grey = control temperature. Black = elevated temperature. DW = dry weight. Treatment groups that do not share a letter are significantly different.**

## Clearance rate

Again, for both species, there was clear inter-individual variability in responses (S3 Fig.). The linear mixed-effects model revealed differences in feeding response depending on exposure and OAW scenario (S3 Fig). For *M. gigas*,  $p\text{CO}_2$  ( $F_{2,18} = 5.8$ ,  $p < 0.05$ ) and exposure time ( $F_{1,66} = 11.3$ ,  $p < 0.001$ ) had significant effects on CRmax (Fig 4.). Intermediate  $p\text{CO}_2$  (~750 ppm) led to ~40% decrease in CRmax in comparison to ambient  $p\text{CO}_2$  conditions (Fig 4 top left). While not statistically significant, there was evidence that suggests an interaction between temperature and  $p\text{CO}_2$  on CRmax (Fig 4 top right). Under control temperature, CRmax was  $1.1 \pm 0.2 \text{ L.h}^{-1}.\text{gDW}^{-1}$  at ambient  $p\text{CO}_2$  but when oysters were exposed to elevated  $p\text{CO}_2$ , CRmax either decreased by ~41% (750ppm) or increased by ~45% (1000ppm). Elevating the temperature led to an increase in CRmax (~91%) under ambient  $p\text{CO}_2$ ; an effect that was then lost under the 750ppm and 1000ppm OA treatments, with CRmax returning to a level comparable with this species held under control temperature and ambient  $p\text{CO}_2$  conditions (Fig 4 top right). After 12 wk, CRmax had decreased by ~41% of the starting clearance rate (Fig 4 bottom left).

For *O. edulis*, CRmax was affected by a combination of temperature and exposure time, but not  $p\text{CO}_2$  ( $F_{1,70} = 11.2$ ,  $p < 0.001$ )(Fig 4 bottom right). Under control temperature, CRmax was not different at 10d, 9 and 12 wk, although there was a reduction in CRmax of ~41% at wk-5. Under elevated temperature, CRmax of *O. edulis* was  $2.9 \pm 0.5 \text{ L.h}^{-1}.\text{gDW}^{-1}$  after 10d exposure (an increase of ~67% over control temperature oysters), but which subsequently dropped back to a rate

comparable to oysters reared under control temperature for the remainder of the study.



**Fig 4. Changes in maximum clearance rate (CR<sub>max</sub>) of: *M. gigas* with pCO<sub>2</sub> treatment (top left), pCO<sub>2</sub> and temperature (top right), exposure duration (bottom left); and *O. edulis* with exposure duration (bottom right). Grey =**



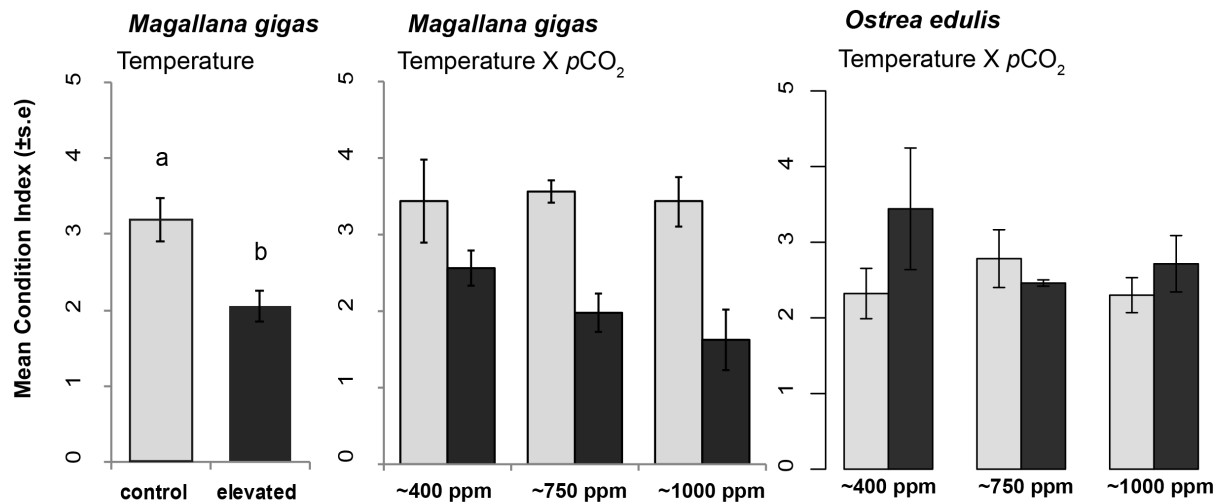
control temperature. Black = elevated temperature. Treatments that do not share a letter are significantly different. DW = dry weight.

## Relationship between the physiological traits

There was no correlation between SMR and CRmax for either *M. gigas* or *O. edulis*.

## Condition index

At the end of the exposure duration, none of the oysters were reproductive. For *M. gigas*, the effects of temperature and pCO<sub>2</sub> were only marginally not significant most likely due to statistical power ( $F_{2,18} = 3.46$ ,  $p = 0.053$ ), but clear trends were apparent. Under ambient temperature, there was no change in mean CI irrespective of pCO<sub>2</sub>, but when temperature was elevated, there was a sustained reduction in mean CI with increasing pCO<sub>2</sub> (Fig. 5b). Considering temperature or pH alone, temperature led to a 40% reduction in CI from  $3.5 \pm 0.2$  to  $2.1 \pm 0.2$  ( $F_{1,18} = 12.5$ ,  $p < 0.01$ , Fig. 5a) but pCO<sub>2</sub> had no effect ( $F_{2,18} = 0.56$ ,  $p = 0.58$ ). In *O. edulis*, neither temperature ( $F_{1,17} = 0.85$ ,  $p = 0.37$ ) or pCO<sub>2</sub> ( $F_{1,17} = 0.10$ ,  $p = 0.902$ ) had any effect on CI, which averaged at  $2.6 \pm 0.1$  (Fig 5c).



**Fig 5. Variations in the condition index of *M. gigas* and *O. edulis* across temperature and  $p\text{CO}_2$  treatments after 12 weeks exposure.** Grey = control temperature. Black = elevated temperature. *M. gigas*: n=4; *O. edulis*: n=4.

## Discussion

Climate change represents an important selection pressure dictating the distribution of species and the functioning of marine ecosystems. Today, there is pressure to understand the effects of multiple stressors on species that provide important ecosystem goods and services (Osborn *et al.*, 2017), and to mitigate any negative impacts in order to ensure the sustainable delivery of these goods and services. Here, following exposure to temperature and  $p\text{CO}_2$  scenarios predicted for the near future, we show species-specific changes in metabolic rate, feeding rate, and condition of two ecologically and economically important species of oysters. Contrary to expectations, non-native *M. gigas* experienced more pronounced negative effects of warming and acidification than the native *O. edulis*, displaying increased metabolic rate under elevated temperature to 20°C, but decreased feeding rate under ~750

501 ppm  $p\text{CO}_2$ , which led to reduced overall condition after 12 weeks. *O. edulis*  
502 appeared relatively unimpacted by future OAW scenarios.

503

## 504 **Metabolism**

505 In marine organisms, the performance of routine activities such as growth,  
506 reproduction, and feeding is supported by the metabolism of oxygen, which is  
507 modulated by environmental conditions such as temperature (Pörtner & Farrell,  
508 2008). Throughout our experiment, the metabolic rate of *M. gigas* was affected by  
509 elevated temperature only. Overall, a  $\sim 3^\circ\text{C}$  temperature increase led to a  $>43\%$   
510 increase in the SMR of *M. gigas*. Similarly, the metabolism of *O. edulis* also  
511 increased with elevated temperature by  $\sim 39\%$ , although unlike *M. gigas*, this  
512 increase coincided with highest  $p\text{CO}_2$  ( $\sim 1000$  ppm) concentrations. This suggests  
513 that both *Magallana gigas* and *Ostrea edulis* display some capacity to withstand  
514 ocean acidification and warming scenarios in the short term, but elevated  
515 temperatures may pose a threat to functioning should increases in metabolism  
516 approach maxima.

517

518 Temperature increasing the metabolism of organisms is common in ectotherms; an  
519 effect previously shown in oysters (Bougrier *et al.*, 1998; Saucedo *et al.*, 2004;  
520 Shpigel *et al.*, 1992) and other bivalves (Artigaud *et al.*, 2014; Matoo *et al.*, 2013).  
521 This is not necessarily problematic if temperature elevations are within the thermal  
522 window of the organism, but ocean warming is expected to push species closer to or  
523 beyond their upper thermal limit with physiological and ecological consequences.  
524 This is especially true for individuals already living close to their upper thermal limit  
525 (Pörtner & Farrell, 2008). In the UK, *M. gigas* is considered to be living in the middle

526 of its thermal range; its capacity to increase metabolic rate under elevated  
 527 temperature supports this assertion. The thermal limits of *O. edulis* are less well  
 528 known, but here, individuals were able to increase metabolic rate under elevated  
 529 temperatures, suggesting some biological scope to withstand the climate scenarios  
 530 predicted for the future.  
 531  
 532 In our study, adult *M. gigas* and *O. edulis* displayed complex responses to variations  
 533 in  $p\text{CO}_2$  conditions, although none significantly changed their SMR, indicating that  
 534 acidification levels tested here ( $\sim 750$  ppm;  $\sim 1000$  ppm  $p\text{CO}_2$ ) might not constitute  
 535 stressful conditions for them. It is likely that these levels are not unusual in coastal  
 536 and estuarine waters, and organisms may well have been subjected to these  $p\text{CO}_2$   
 537 levels before (Hales *et al.*, 2016). However, the metabolic response of bivalves to  
 538 elevated  $p\text{CO}_2$  appears species and population-specific. Several other studies  
 539 examining the effect of  $p\text{CO}_2$  on respiration rate in bivalves at concentrations  
 540 equivalent to those tested here also revealed no change in SMR (e.g. *Crassostrea*  
 541 *virginica* (at 800 ppm - Matoo *et al.*, 2013), Mediterranean mussels *Mytilus*  
 542 *galloprovincialis* (at  $\sim 1090$  ppm - Gazeau *et al.*, 2014), and scallops, *Pecten*  
 543 *maximus* (at either 750 ppm and 1140 ppm - Sanders *et al.*, 2013)). Pronounced  
 544 changes in respiration rates can be shown when  $p\text{CO}_2$  levels greatly exceed those  
 545 tested here (e.g. increasing in *C. virginica* (at 3500 ppm - Beniash *et al.*, 2010) and  
 546 *Mytilus edulis* (at 1120 ppm and 2400 ppm - Thomsen & Melzner, 2010), but  
 547 reducing in *Ruditapes decussatus* (between 1698 ppm and 4345 ppm - Fernández-  
 548 Reiriz *et al.*, 2011)). It is argued that increases in metabolic rates allow individuals to  
 549 maintain their internal acid-base balance and maintain routine physiological activities,  
 550 such as biomineralization (Melzner *et al.*, 2009; Pörtner & Farrell, 2008) although the

conditions used to stimulate these changes greatly exceed  $p\text{CO}_2$  concentrations predicted for the next 80 years.

Previously, interactive effects of  $p\text{CO}_2$  and temperature on metabolism have been shown (e.g. Lannig *et al.*, 2010, at ~1480 ppm and 20°C or 25°C); an effect reinforced in *O. edulis* in this study which showed that elevated temperature could compensate for the decreasing trend in SMR under elevated  $p\text{CO}_2$  (~1000 ppm) and lead to an overall increase in SMR. Increasing metabolic rate is energetically expensive. This may be a physiological response developed to cope with stressful conditions in the short-term but could also be an involuntary change caused by a speed-up of biochemical reactions. Irrespective of the mechanism, this suggests a higher energy demand necessary for the maintenance, active metabolism, and overall survival of oysters. However, long-term elevation in SMR may not be sustainable for organisms due to the added energetic costs, particularly if left uncompensated, unless they become adapted over multiple generations.

## **Clearance rate**

In the literature, the terms feeding rate, ingestion rate, clearance rate, and filtering rate are often used in concomitance or interchangeably (e.g. Coughlan, 1969; Fernández-Reiriz *et al.*, 2011; Sanders *et al.*, 2013). All are related to the amount of particles or the volume of water being processed over time. Previous studies have shown that respiration and feeding in oysters are related (Giomi *et al.*, 2016; Haure *et al.*, 2003; Haure *et al.*, 1995). Higher metabolism leads to higher energetic demands, commonly met through enhanced food consumption. Here however, contrary to predictions, no relationships between respiration and feeding rates were

found for either species. Nevertheless, in our study, the clearance rate of *M. gigas* followed an increasing trend under elevated temperature, particularly at ambient and intermediate  $p\text{CO}_2$  (Fig 5.), suggesting a mechanism working towards enhanced food acquisition and energy supply. While increased feeding activity with temperature has been previously shown in several species of mollusc (e.g: *O. edulis* (non-linear increase from 10°C to 30°C - Haure *et al.*, 1998, and references therein), *M. galloprovincialis* (from 12°C to 18°C - Kroeker *et al.*, 2014), and *Mytilus chilensis* (between 12°C and 16°C - Navarro *et al.*, 2016)), it was observed in *O. edulis* in this study only after 10 days of exposure, following which clearance rates returned to control levels. This suggests an initial acclimation response to experimental conditions rather than a longer-term response to the treatment.

Elevated  $p\text{CO}_2$  reduced the clearance rate of *M. gigas* by up to 40%; an effect not observed in *O. edulis*. There is a burgeoning literature on the effects of elevated  $p\text{CO}_2$  on the feeding behaviour and clearance rate of juvenile and adult bivalves, both of which are increasingly recognised as potential key physiological traits that govern an organisms' responses to ocean acidification (Vargas *et al.*, 2015).

Although feeding is an energetically expensive process (Pörtner *et al.*, 2004), it has the potential to alleviate the negative effects of ocean acidification by providing the required additional energy to overcome the increased cost of metabolism. Indeed, several studies have shown that high food availability can counteract the effects of acidification on molluscan larvae and juveniles (Hettinger *et al.*, 2013a; Sanders *et al.*, 2013; Thomsen *et al.*, 2012). However, elevated  $p\text{CO}_2$  has also been shown to negatively impact on the clearance and ingestion rates of several species of molluscs as found here for *M. gigas*. Juveniles of the mussel *Perumytilus purpuratus*

decreased their clearance rates by up to 70% under similar  $p\text{CO}_2$  levels (at 700 ppm and 1000 ppm - Vargas *et al.*, 2015). Elevated  $p\text{CO}_2$  to 1000 ppm also led to reduced clearance rate and absorption efficiency in *M. chilensis* (Navarro *et al.*, 2016), and to a weak decrease in feeding rates in *M. galloprovincialis* at 1200 ppm (Kroeker *et al.*, 2014). Additionally, more extreme  $p\text{CO}_2$  levels have also been linked to reduced clearance and ingestion rates in juveniles of the clam *R. decussatus* (between 1698 ppm and 4345 ppm - Fernández-Reiriz *et al.*, 2011). Impairment of filtration and feeding can prevent organisms from resisting ocean acidification or compensating for its effects, with subsequent starvation leading to increased mortality within the population. In accordance with our results for *O. edulis*, no marked effects of elevated  $p\text{CO}_2$  on clearance rate were recorded in *P. maximus* (at levels up to 1140 ppm - Sanders *et al.*, 2013). These results reinforce the idea that responses to acidification conditions are not only species-specific, but also dependent on the range and number of  $p\text{CO}_2$  levels considered.

## Condition index

The higher metabolic costs associated with increased respiration rates under future OAW conditions, particularly in *M. gigas*, were not compensated for by added energy through enhanced feeding. However, added energetic demands can also be met by other trade-offs with calcification, reproduction, and growth of somatic tissues.

Condition Indices (CI) are recognised as useful tools to evaluate the overall status and health of bivalves (Knights, 2012), and reflect their ability to withstand adverse environmental conditions (Marin *et al.*, 2003). Stressful environmental conditions requiring significant energetic expenditure result in low CI in bivalves over time

(Orban *et al.*, 2002). Here, the CI of *M. gigas* was negatively impacted by elevated temperature but not elevated  $p\text{CO}_2$ , an effect also seen for the mussel *M. edulis* (Mackenzie *et al.*, 2014). Our results for  $p\text{CO}_2$ -exposed individuals are in contrast to those of Lannig *et al.*, (2010) on *M. gigas* who recorded a decrease of ~20% in CI between control individuals and those exposed to elevated  $p\text{CO}_2$ . However, similar decreases in CI with elevated temperature were recorded in several other bivalves (Gabbott & Walker, 1971; Hiebenthal *et al.*, 2012; Shpigel *et al.*, 1992). Bivalves have the capacity to reallocate energy reserves by reabsorbing somatic tissues and gonads to sustain routine maintenance when needed. Declines in CI usually suggest depletion of these reserves and are often associated with long-term stressful conditions (Lannig *et al.*, 2010) or alterations in energy budget (Melzner *et al.*, 2009).

As reduced condition index is associated with depletion of energetic reserves, it suggests that the long-term costs associated with increased metabolism in *M. gigas* were met by a reallocation of reserves from somatic and gonadal tissues to sustain maintenance and insure survival. While no mortality of *M. gigas* occurred during the experiment, the lack of acclimation in respiration and clearance rates responses after 12 weeks exposure suggests that, if left uncompensated, the added metabolic costs could compromise survival once all somatic and gonadal reserves are depleted.

The CI of *O. edulis* was unaffected by any of the treatment conditions, suggesting that the experimental environmental conditions were not equally experienced by both species, and *M. gigas* only may be stressed. A potential explanation for the maintenance of *O. edulis* CI when exposed to the elevated temperature and ~1000 ppm  $p\text{CO}_2$  treatment despite increased metabolic rates is that its sustained clearance



rates provided sufficient energy supply to compensate for the additional metabolic costs over the 12 weeks. Nevertheless, exposure beyond the 12 week period of this study might produce *O. edulis* displaying lowered CI from longer-term accumulated and uncompensated energetic costs.

## **Conclusion:**

This study has shown that two important physiological traits of oysters are affected by warming and/or acidification, however the responses appear species-specific. Due to logistic limitations inherent to the OAW system used during the experiment, the sample size for each species was limited to n=4 per treatment and as such, there was high variability in the responses recorded, which led to lack of statistical power for the analysis. Yet despite this, clear biological effects were apparent. If anthropogenic CO<sub>2</sub> emissions continue to rise and temperatures continue to increase, increased metabolic cost to oysters are predicted. *Magallana gigas* in particular may find it difficult to meet these costs due to decreased feeding activity at ~750 ppm pCO<sub>2</sub> levels. Non-native and invasive species are often more resilient to environmental fluctuations and other biotic or abiotic stressors, yet in oysters sampled from wild Plymouth populations, *M. gigas* was more negatively impacted by the OAW scenarios tested than its native counterpart, *O. edulis* – which contradicted our initial predictions. The non-native oysters had elevated metabolism, reduced feeding, and decreased condition, signs that it could not cope well with the warming and acidification conditions. Krasso *et al.* (2008) demonstrated that differences exist with respect to abiotic environmental tolerances of extreme physical conditions between exotic and native oyster species, with the native species able to withstand harsher environmental conditions. This was also recently observed in Brazil, where

676 the native *Crassostrea brasiliana* was more tolerant to high temperatures than the  
677 non-native *M. gigas* (Moreira *et al.*, 2017). However, it should be noted that, although  
678 here only two factors were tested, the interaction of multiple environmental drivers  
679 has been shown to influence the sensitivity of organisms to a single specific factor  
680 (Parker *et al.*, 2017a; Parker *et al.*, 2017b).

681

682 Due to poorer performance and condition of individual *M. gigas*, as found here,  
683 warming and acidification may threaten populations maintenance and functioning,  
684 degrading the provision of ecosystem services such as erosion control, improved  
685 water quality, and fisheries from unharvested wild beds, while reducing aquaculture  
686 productivity at designated aquaculture sites. The latter is especially important in the  
687 UK where harvest of cultured *M. gigas* populations constitutes 90% of the oyster  
688 aquaculture production, worth an estimated £10.14 million annually (Humphreys *et*  
689 *al.*, 2014). Additionally, reduced clearance rates of *M. gigas* under OAW may have  
690 important ecological impacts by limiting their ability to reduce turbidity and improve  
691 water quality. Similar concerns have been expressed regarding the fate of waste  
692 bioremediation service by mussels under future ocean acidification, as their filtration  
693 rates might be negatively impacted (Broszeit *et al.*, 2015). Wild unharvested oyster  
694 beds consisting in majority of *M. gigas* might see their surrounding water quality  
695 diminish, with negative consequences for further associated ecosystem services  
696 such as allowing for recreational use and promoting the maintenance of submerged  
697 vegetation. In contrast, it appears that under future OAW corresponding to the levels  
698 tested in this study, *O. edulis* will be able to continue delivering its important bio-  
699 filtration service, and consequently the provision of improved water quality will  
700 remain secure, if abundances recover and beds become functional again.

Such findings are of importance in terms of species ecological status, population conservation, and management measures. Oyster-related ecosystem services are mostly associated with ‘reef’ formations, which would require high recruitment and abundant populations (Herbert *et al.*, 2012). As such, further efforts to promote the restoration of native *O. edulis* beds should be pursued, and efforts to eradicate *M. gigas* populations may be reconsidered, in order to secure not only food provision, but also good water quality and associated beneficial ecosystem services in the future from functional populations of both species. However, ecological and economic trade-offs will need to be considered carefully, as the delivery of some of these ecosystem services from wild populations (food provision vs water quality) may be at odds given their opposing effects on oyster abundances.

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1117

## 1118 **Supporting information**

1119 **S1 Table. Physical and chemical characteristics of seawater in the six**  
 1120 **experimental treatments for *Magallana gigas* and *Ostrea edulis*.** Presented as  
 1121 mean values over the duration of the experiment  $\pm$  standard deviation (s.d.). T=  
 1122 temperature, S= salinity,  $\Omega_a$ = saturation state of aragonite,  $\Omega_c$ = saturation state of  
 1123 calcite.

1124

1125 **S1 Fig. Temperature (left) and pH (right) data within the mesocosm set-up**  
 1126 **throughout 3-month exposure of a) *Magallana gigas* and b) *Ostrea edulis***  
 1127 **exposed to two temperature levels: control (~16.5°C - blue); elevated (~20°C -**  
 1128 **red) three  $p\text{CO}_2$  levels: Ambient (~400 ppm - white), ~750 ppm (yellow), ~1000**  
 1129 **ppm (black).**

1130

1131 **S2 Fig. Changes in standard metabolic rate (SMR) of *M. gigas* (top) and**  
 1132 ***O. edulis* (bottom) over 12 weeks exposure to temperature and  $p\text{CO}_2$**   
 1133 **combinations.** Grey = control temperature. Black = elevated temperature. DW = dry  
 1134 weight.

1135 **S3 Fig. Changes in maximum clearance rate (CR<sub>max</sub>) of *M. gigas* (top) and**  
1136 ***O. edulis* (bottom) over 12 weeks exposure to temperature and pCO<sub>2</sub>**  
1137 **combinations.** Grey = control temperature. Black = elevated temperature. DW = dry  
1138 weight.

1139

1140 **Highlights:**

- 1141 • Acidification and warming negatively impacted the physiology of *Magallana*  
1142 *gigas*
- 1143 • *Ostrea edulis* appeared unaffected by the treatment conditions
- 1144 • Efforts to promote the restoration of native *O. edulis* beds should be pursued
- 1145 • Efforts to eradicate *M. gigas* populations may need to be reconsidered